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1. *SALMONELLA*/MICROSOME ASSAY

A. Expanded Bright WSC Activities[1,2]

To confirm and extend results obtained last year,[3] a new set of cigarettes containing expanded bright tobacco was fabricated specifically to examine the effects of cylinder reordering with and without blow-box equilibration on ET (NH_3/CO_2 expansion process) and carbamate ET (ammonium carbamate expansion process) WSC activity. The results (which included a statistical analysis conducted by J. E. Tindall) were as follows.

WSC	TA98 Activity[1,2]
ET (blow-box equilibrated)	1300
ET	1252
Carbamate ET	1256
Carbamate ET (blow-box equilibrated)	1106
Westab Feedstock (Control)	954
DET (MC)	692

1) TA98 Activity (revs/mg of WSC): mean of determinations conducted on four separate preparations of each WSC in the presence of microsomes.

2) Samples not within the same bracket are different, $p<0.05$.

The overall results were consistent with those previously reported; *i. e.*, tobacco expanded by the two processes which lead to increased levels of filler nitrogen (ET and carbamate ET) yielded WSCs of increased activity (relative to their unexpanded control WSC). In contrast, tobacco expanded by the liquid CO_2 process (DET), which does not affect the level of filler nitrogen, yielded a WSC of reduced activity.[3] The blow-box equilibration treatment had no effect on ET WSC activity. However, for carbamate

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ET, the blow-box equilibration treatment significantly reduced WSC activity. The reason(s) for the latter result is obscure, but it may be that the reduction in filler nitrogen which accompanied blow-box equilibration of the carbamate ET is associated with this phenomenon. (Blow-box equilibration of ET slightly increased filler nitrogen.)

B. Denitrated RL WSC Activities[4]

Tests were begun on a series of WSCs derived from nine different 100% RL cigarettes which represent each of the denitration processes currently being used or evaluated at PM. In the first experiment, which involved tests in strains TA98 and TA100 ± microsomes, no unusual features were observed in the activity profiles of any of the samples. A second experiment is in progress to establish the relative activities of the nine samples based on testing four replicate WSC preparations from each cigarette type in TA98 (+ microsomes).

2. YEAST MITOTIC GENE CONVERSION ASSAY[5]

A. WSC Fractions (with 6908)

To extend our knowledge about base fraction activity,[6] a set of burley WSC fractions was tested: the basic fraction and the methanol (MeOH) and ether eluates obtained from alumina column chromatography of the bases.[7] The results showed that: the MeOH eluate contained the bulk of the basic fraction activity; the ether eluate was only weakly active; and the MeOH eluate was less active than the basic fraction. Except for the latter result, these conclusions were similar to those obtained in the *Salmonella*/microsome assay.[7] Further experiments are planned.

B. Activity of Nicotine

It was concluded that nicotine was inactive in this assay based on the absence of a clear dose-response relationship. Addition of nicotine to 2A1 WSC (low alkaloid Kentucky reference) was found to have no effect on WSC activity.

C. Denitrated RL WSC Activities

Tests are in progress on the RL WSCs described above (1. B.). In the first experiment, no unusual features or differences were observed on the dose-response curves of any of the nine WSCs.

3. L5178Y THYMIDINE KINASE (TK) MUTATION ASSAY

Evaluation of $TK^{-/-}$ Mutants[8]

The TK enzyme assays were completed on a series of twelve suspected $TK^{-/-}$ mutants (induced spontaneously, with positive control

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compounds, or with WSCs) selected in the presence of trifluorothymidine. All of the mutants were found to be deficient in TK activity. The objective of these studies has now been accomplished: to place the assay on a firm molecular basis by confirming that the mutants obtained are truly $TK^{-/-}$ based on selective growth and toxicity studies and measurements of TK activity. A Special Report will be written to document these conclusions.

B. Standard *versus* Modified Cloning Procedure[9]

As alluded to last month, higher spontaneous background and positive control compound activities recently observed have been a cause of concern.[6] The results of an experiment conducted to investigate several possible reasons for the higher responses showed that they were not attributable to: the microsomes; the positive control compounds; or the solvent. Further experiments are planned.

C. Filler Precursors of WSC Activity--LTF Variants[10]

Experiments are in progress to confirm our previous observations regarding the removal of LTF-IIIA filler components.[6] Included in these studies is a comparison of LTF-IIMF *versus* LTF-IIJF, which differ because the latter filler contains urea and color.

4. BHK CELL TRANSFORMATION[11]

The evaluation of our first cell clone was begun by measuring its response(s) to a positive control compound (ethylmethanesulfonate) in growth media containing different types of sera. No transformation was observed under any of the conditions tested. It was observed, however, that freezing the sera (rather than storing them in the refrigerator) led to more reproducible cell generation times in successive passages. Evaluation of a second cell clone is in progress.

5. REFERENCES

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